Susceptibility Testing for Fastidious Organisms: CLSI M45-A3 update

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October 24, 2015

History

- CLSI has provided testing and interpretive criteria for common organisms
- Labs occasionally need to test “infrequently isolated or fastidious” species
- In absence of guidelines, labs have used various methods and applied breakpoints from other groups of organisms
- There is a need to provide better guidance for use of standard methods for these unusual species
- M45-A Published 2006; M45-A2 2010; M45-A3 2015
- Janet Hindler and Jim Jorgensen spearheaded the project

Update - M45 A3

- Forward - statement that commercial devices that are FDA approved may be suitable for testing.
- Includes antibiotic gradient method or the Etest
- However, this document addresses only generic reference procedures that can be used routinely or to evaluate commercial devices

The M45-A2 organisms

- Abiotrophia and Granulicatella spp
- Aeromonas hydrophila complex
- Bacillus spp.
- Campylobacter jejuni/cali
- Corynebacterium spp.
- Erysipelothrix rhusiopathiae
- HACEK group
- Lactobacillus species
- Leuconostoc species
- Listeria monocytogenes
- Moraxella catharralis
- Pasteurella multocida
- Pediococcus species
- Vibrio species
- Helicobacter pylori

New organism in M45-A3

- Aerococcus
- Additional Bacillus spp
- Additional genera to Coryneforms
- Gemella (3 species)
- Lactococcus spp
- Micrococcus spp
- Rothia mucilaginosa

Introduction - additions

- Updated taxonomy
- Resistance mechanisms for all groups have been moved from the introduction to their respective tables
- Added list of abbreviations and acronyms
- More information on QC strains
- New antimicrobial agents added throughout
Traditional factors used for MIC breakpoint definitions

- MIC distribution in wild type
- MICs with known resistance mechanisms
- Pharmacokinetics and pharmacodynamics
- Monte Carlo simulations – dose vs maintenance of drug level vs MIC
- Clinical and bacteriological response from clinical trial data
- Animal models

Challenges of the “orphans”

- Relatively few strains available
- Resistant strains may be rare
- No clinical trials
- Most clinical data from case reports
- Lab data from centers doing surveys and studies
- Guidelines for breakpoints cannot be as stringent as standards for common species

M45 A3– Format for tables

- Box - Testing conditions - medium, inoculum, incubation
- Box - Minimum QC recommendation
- Box - Agents to consider for primary testing
- General comments – e.g. very fastidious, require CO2
- Antimicrobial agents – S-I-R – comments
- Supplemental information - resistance, reasons for testing, derivation of interpretive criteria

Abiotrophia and Granulicatella

Minimal changes

- Test as S. pneumoniae except supplement with 1ug/ml pyridoxal HCl
- QC S. pneumoniae; interpretive criteria adapted from M100 for Streptococcus.
Comment that addition of gentamicin is recommended for combination therapy for endocarditis
- Very fastidious - requires cysteine or pyridoxal; may grow as satellite colonies around staph
- May demonstrate diminished susceptibility to pcr: fluoroquinolone-resistant strains reported
**Aerococcus spp. (new)**

- **Testing conditions**
  - CAMHB with LHB; Direct colony suspension; Incubate 20-24h in CO2 incubator at 35°C.
  - QC - S. pneumoniae ATCC 49619
- **Primary testing:** PCN, ceftriaxone, vancomycin
- **Aerococcus spp.** are usually susceptible to β-lactams, and vancomycin. Resistance has been described to the fluoroquinolones, associated with mutations to the quinolone resistance determining region (QRDR) of gyrA or parC. *Aerococcus sanguinocola* and *Aerococcus viridans* isolates commonly display resistance to levofloxacin.
- Testing of isolates from normally sterile sources (blood cultures) may be warranted.

**Aeromonas spp**

- **Includes members of A. caviae complex, A. hydrophila complex, and A. veronii complex (17 valid species)**
- CAMHB, direct colony suspension, 35°C ambient air, DD-16-18h, BMD 16-20h
- Pleisomona deleted and transferred back to M100 because of similarity to Enterobacteriaceae
- Testing usually for extra intestinal sites

**Aeromonas hydrophila infection**

- **Acute Post op infection with Aeromonas from leech therapy**
- 5 male patients with trauma injuries
- Post op - venous congestion
- Medical leeches applied
  - Passive bleeding after bite
  - Superficial skin perfusion after bite
- Anti-coagulant effect of saliva
- All developed infection with A. hydrophila
- Leech guts are colonized with Aeromonas

**Bacillus spp. - not B. anthracis**

- **Added fluoroquinolones for primary testing**
- **Testing conditions** - same as staph
- QC - S. aureus
- Reasons for testing normally sterile sites esp in immunodeficient pts
- Beta-lactamase testing is unreliable
- Interpretive criteria derived from M100 Staphylococcus
- Do not test oxacillin

**Bacillus infections and new genera added**

- Nosocomial central catheter-related bacteremia following cholecystectomy in an immunocompetent patient
- B. cereus bacteremia in preterm neonate (review)
- Bacillus spp. and hematological malignancies - 3.4% of episodes
- New genera: Brevibacillus, Lysinibacillus, Paenibacillus, Sollbacillus, Sporolactobacillus
- Recovered from UTI, endocarditis

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(Schnabl et al, Arch Orthop Trauma Surg 2000)

(Ozkocaman et al, JHI 2006 64:169)
**Bacillus spp. susceptibility to antimicrobial agents**

- **B. cereus group**
  - Most R to pen; 2 of 29 R to cipro; R to erythro sporadic;
  - 1 strain R to doxy

Ikeda study - BSIs - 29 cases
- 65% r to clinda, 10.3% r to levo
Injection 3 drug users - Canada - RX Vanco

- Other Bacillus spp - including B. subtilis, B. pumilus, B. firmus. Most S to cipro, many to pcn, most S to erythro

(Citron et al JCM 2006 44:3814)

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**Campylobacter jejuni/coli**

- Added tetracycline
- Clarified incubation conditions for disk and MIC tests
- Test conditions - CAMHB + LHB
- Incubation - 42°C for 24h. Microaerophilic atmosphere. Use gas generating packets or compressed gas mixture.
- Resistance known to occur with erythro, but more problematic with cipro.
- Use of disks - no zone as resistance screen for cipro and erythro. Any zone of inhibition requires MIC determination.

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**Corynebacterium and other coryneforms**

- Modified breakpoints for pcn - <0.12; 0.25 - 2; ≥4
- Clarified scope of other GFRs
- Test conditions - CAMHB + LHB
- QC - S. pneumoniae
- Some strains fastidious - require blood supplementation
- Removed imipenem, added meropenem criteria
- Interpretive criteria for pcn and erythro based on MIC distributions from testing large numbers of isolates: ceph from Strep spp, linezolid from Enterococcus; remainder from Staph (M100)
  - Do not test oxacillin
  - Report resistant results after 24h; if B-lactams appear sens, reincubate for 48h

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**New coryneforms**

- Arcanobacterium
- Arthrobacter
- Brevibacterium
- Cellulomonas
- Cellulosimicrobium
- Dermabacter
- Leifsonia
- Microbacterium
- Oerskovia
- Rothia spp*
- Trueperella
- Turicella

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**Recent reports of infections with Corynebacterium spp**

- Septic arthritis - C. striatum, C. kutscheri
- Encrusted cystitis - C. urealyticum
- Toxigenic C. ulcerans - mimics diphtheria
- Granulomatous mastitis - C. amycolatum, C. accolens, C. striatum, C. kroppenstedtii
- Exacerbation of COPD - serious resp inf - C. striatum (3 deaths with pure cult)
- Pancreatic abscess - Corynebacterium coyleae mimicking malignant neoplasm.
- Urinary tract infection - Corynebacterium pseudogenitalium

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**Listeria monocytogenes**

- C. amycolatum, C. jeikeium do not always show resistance in BMD after 24h
Gemella spp.
- Test conditions same as viridans group of streptococci
- Primary drugs - ceftriaxone, pcn, vancomycin
- Combined therapy with pcn plus genta for endocarditis
- Most isolates require 48h incubation
- All species tend to very susceptible to all antimicrobial agents

(H)ACEK organisms
- Aggregatibacter new genus name for Actinobacillus actinomycetemcomitans and Haemophilus aphrophilus
- Testing conditions - CAMHB + LHB
- Incubation - 35C, CO2, 24-48h
- QC - S. pneumoniae
- Growth characteristics - very fastidious, most will grow on BAP or CHOC in CO2. Some will not grow in supplemented broths. Do not attempt to interpret results if insufficient growth in control well.
- Etest more reliable than BMD
- Routine testing for B-lactamase recommended

Helicobacter pylori
- Imported from M100
- Agar dilution method using 2 week old sheep blood
- Inoculum - turbidity = to No. 2 McFarland standard
- Incubation - microaerobic environment
- Test group - clarithromycin
- Metronidazole does not work in microaerobic environment. Some have suggested Etest under anaerobic conditions for 24h.
- Reasons for testing - clinical failure

Lactobacillus spp
- Added breakpoints for dapto and linezolid
- Added I and R breakpoints for imipenem
- Added meropenem breakpoints
- Modified clinda and vanco breakpoints
- Expanded species intrinsically R or S to vancomycin
- Test conditions- CAMHB + LHB
- 35 C with 5% CO2 24-48h
- Test PCN, ampicillin
- L. casei group are intrinsically Van-R
- L. acidophilus group - Van-S

Lactobacillus gasseri
- Elderly lady admitted from nursing home with urosepsis
- Several blood culture sets yielded large GPB
- S to vanco, catalase-neg
- ID by 16S rDNA seq.

Lactobacillus Bacteremia
- Vancomycin-resistant group
  - L. rhamnosus 53.5%
  - L. fermentum 14%
  - L. casei 14%
- Vancomycin-susceptible
  - Other species 18.6 %
    - L. jensenii
    - L. salivarius
    - L. gasseri
- All have PCN MICS ≤ 4 ug/ml

Salminen et al CID 2006:42
**Lactococcus spp.**
- Conditions for testing, same as viridans strep.
- Abx for primary testing - Ctx, clinda, erythro, pcn or ampi, vanco
- L. garvieae is intrinsically R to clinda. R also reported in L. lactis ssp. Lactis. Tetra R also reported.
- Risk factors - exposure to raw fish
- Catheter associated bacteremia, neonate UTI, subdural empyema, neck abscess resembling CA

**Leuconostoc**
- Added comment for adding gent to pcn or amp for serious infections
- Deleted gentamicin interpretive criteria
- Testing conditions - CAMHB + LHB
- QC - S. pneumoniae
- Incubation - ambient, 20-24h
- Test pcn, amp; intrinsically vanco-R
- Interpretive criteria from staph (GM), Enterococcus for other drugs

**Listeria monocytogenes**
- Testing - CAMHB - LHB
- QC - S. pneumoniae
- Antimicrobials - ampicillin, penicillin, TMX
- Added meropenem interpretive criteria
- Revised TMX interpretive criteria to include susceptible only
- Intrinsically R to ceps
- R to pcn/ampi has not been described

**Micrococcus spp**
- Testing conditions same as for staph
- Primary testing - pcn and vanco
- Testing data may be applied to orgs previously included in Micrococcus
  - Kocuria
  - Nesterenkonia
  - Dermacoccus
  - Kytococcus
- Resistance to beta-lactams and erythromycin has not been reported
- Recovered from multiple positive blood cultures, prosthetic devices, endocarditis

**Moraxella catarrhalis**
- Deleted cefaclor interpretive criteria
- Resistance to amox-clav and cephalosporins has not been reported, so interpretive criteria defined for S only
- Testing - CAMH, disk or MBD
- QC - S. aureus, E. coli
- Primary antimicrobials - amox-clav, cefuroxime, TMX
- Resistance to tetracyclines and TMX has been reported.
- Routine testing is not recommended

**Pasteurella species**
- Clarified incubation conditions
- CAMHB + LHB
- MHA w/5% SB for disk diffusion
- QC - S. pneumoniae BMD; S. aureus for disk
- Fastidious - requires blood
- Rare isolates B-lactamase pos; pcn MICs >0.5
- Testing not needed for bite isolates; testing from normally sterile sites and respiratory infections
- Interpretive criteria based on MIC distributions from large numbers of isolates from human animal bite infections
- Testing S. aureus 25923 on BMHA - use M100, Table 3 ranges
Pasteurella infections not associated with bites
- Pneumonia and septicemia in immunocompetent patient without any evidence of cutaneous lesion - only exposure to dog.
- Septic shock, sinusitis, pneumonia in 20 yo female with close contact with cat, but only minor scratches on arm
- Other reports of meningitis, peritonitis, osteoarticular infections, endocarditis

Pediococcus
- Deleted interpretive criteria for genta
- Added recommendation for genta combination rx with pcn, ampi
- Test conditions - CAMHB + LHB
- Incubate - 35C ambient 20-24h
- QC - S. pneumoniae
- Intrinsically vanco-resistant
- Test isolates from normally sterile sites
- Interpretive criteria adapted from Enterococcus, M100

Rothia mucilaginosa
- Testing Conditions same as viridans strep, Primary testing - pcn, vanco
- Resistance to B-lactams, clinda, erythro, and fluoroquinolones has been reported
- Interpretive criteria derived from staph
- Recovered from bacteremia
  - Risk factors - neutropenia, leukemia
  - Common source - mucositis, gut translocation, catheter related
  - Also - meningitis, endocarditis, pneumonia, osteomyelitis

Vibrio spp. including V. cholera
- Added doxycycline for primary testing
- Revised interpretive disk zone diameters for cefepime, imipenem meropenem
- Testing conditions - CAMHB for broth microdilution; MHA for disk diffusion
- Direct colony suspension in saline
- Test isolates from extra intestinal sites
- Test teta, doxy, cefotaxime, fluoroquinolones
- Usually resistant to PCN, older cephs, sulfonamides
- Interpretive criteria adapted from Enterobacteriaceae

Vibrio vulnificus infections
- Case 1 - 27 yo man struck by lightning while windsurfing. Found pulseless in water and was resuscitated. Developed resp failure, nec fasc, pos blood cultures. Treated with abx and recovered.
- Case 2 - 43 yo Asian oyster shucker with redness, swelling and photophobia in R eye. DX - corneal ulcer with pos culture
- Case 3 - 46 yo man with abdominal pain, nausea, chills, bullous lesions on legs. Developed DIC, and cultures from legs grew V. vulnificus. He ate raw oysters 3 days previously.
- Case 4 - 32 yo woman with HIV, HepC with cirrhosis, presented with fever, chills, productive cough, and red spots on her extremities and buttocks. Blood cultures pos for V. vulnificus. Treated with abx and recovered.
Potential agents of bioterrorism

- Includes B. anthracis, Y. pestis, B. mallei, B. pseudomallei, F. tularensis, and Brucella spp.
- Broth microdilution media
- Incubation conditions for each
- Agents to test
- Interpretive criteria
- QC strains for each
- Precautions - recommend BL-2 practices
- Notify Public Health dept and send isolate for additional testing

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<thead>
<tr>
<th>Table 17. Summary of Testing Conditions and Quality Control Recommendations for Infrequently Isolated or Fastidious Bacteria</th>
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<tbody>
<tr>
<td><strong>Agent</strong></td>
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<tr>
<td>B. anthracis</td>
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<tr>
<td>Y. pestis</td>
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<td>B. mallei</td>
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<td>B. pseudomallei</td>
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<td>F. tularensis</td>
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<td>V. cholerae</td>
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<td>Vibrio spp.</td>
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<td>P. pseudomonas</td>
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<td>P. aeruginosa</td>
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<td>S. aureus</td>
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<td>E. coli</td>
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Conclusion

- This document provides guidelines for testing these unusual, fastidious organisms
- CLSI welcomes comments, questions, suggestions
- Especially important for this document because of the derivation of information
- www.clsi.org